NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF METHYLEUGENOL

(CAS NO. 93-15-2)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

July 2000

NTP TR 491

NIH Publication No. 00-3950

National Toxicology Program

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

K.M. Abdo, Ph.D., Study Scientist
G.M. Blumenthal, Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D
R.A. Herbert, D.V.M., Ph.D.
R.R. Maronpot, D.V.M.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
K.L. Witt, M.S., Integrated Laboratory Systems, Inc.

Southern Research Institute
Conducted 14-week studies, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
A.G. Manus, D.V.M.
R.B. Thompson, D.V.M., Ph.D.

Battelle Columbus Laboratories
Conducted 2-year studies, evaluated pathology findings

M.R. Hejtmancik, Ph.D, Principal Investigator
J.D. Johnson, Ph.D.
M.J. Ryan, D.V.M., Ph.D.
J.D. Toft II, D.V.M., M.S.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
E.T. Gaillard, D.V.M., M.S.

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
K.P. McGowan, M.B.A.
M.A. Mauney, M.S.
N.G. Mintz, B.S.
J.T. Scott, M.S.

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats and mice (12-14 May 1998)

J.C. Seely, D.V.M., Chairperson (Rats)
PATHCO, Inc.
P.K. Hildebrandt, D.V.M., Chairperson (Mice)
PATHCO, Inc.
J. Everitt, D.V.M., M.S.
Chemical Industry Institute of Toxicology
E.T. Gaillard, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
J.R. Hailey, D.V.M.
National Toxicology Program
G.C. Hard, BVSc., Ph.D., DSc.
American Health Foundation
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J. Mahler, D.V.M.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
M.J. Ryan, D.V.M., Ph.D.
Battelle Columbus Laboratories
R.C. Sills, D.V.M., Ph.D.
National Toxicology Program
S. Streett, V.M.D.
C.S. Streett & Associates
J.D. Toft II, D.V.M., M.S.
Battelle Columbus Laboratories
H.J. White, M.D.
Consultant

Biotechnical Services, Inc.

Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
J.A. Gregan, M.A.
L.M. Harper, B.S.
A.M. Macri-Hanson, M.A., M.F.A.
D.C. Serbus, Ph.D.
W.D. Sharp, B.A., B.S.
D.P. Shaw, B.A.
S.M. Swift, B.S.

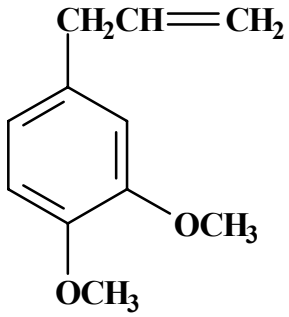
Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

CONTENTS

ABSTRACT



METHYLEUGENOL

CAS No. 93-15-2

Chemical Formula: $C_{11}H_{14}O_2$ Molecular Weight: 178.2

Synonyms: 4-Allyl-1,2-dimethoxybenzene; 4-allylveratrole; 4-allyl-3,4-dimethoxy-benzene; 1,2-dimethoxy-4-allylbenzene; 3,4-dimethoxyallylbenzene ENT 21040; 1-(3,4-dimethoxyphenyl)-2-propene; eugenol methyl ether; 1,3,4-eugenol methyl ether; veratrole methyl ether

Methyleugenol is used as a flavoring agent in jellies, baked goods, nonalcoholic beverages, chewing gum, candy, pudding, relish, and ice cream. It is also used as a fragrance in perfumes, creams, lotions, detergents, and soaps. Methyleugenol has also been used as an insect attractant in eradication programs and as an anesthetic in rodents. Methyleugenol was nominated for testing because of its widespread use and because of its structural resemblance to safrole, a known carcinogen, and isosafrole and estragole. Male and female F344/N rats and B6C3F$_1$ mice received methyleugenol (approximately 99% pure) in 0.5% methylcellulose by gavage for 14 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and mouse peripheral blood erythrocytes.

14-WEEK STUDY IN RATS

Groups of 9 or 10 male and 10 female F344/N rats were administered 0, 10, 30, 100, 300, or 1,000 mg methyleugenol/kg body weight in 0.5% methylcellulose by gavage 5 days per week for 14 weeks. A water control group of 10 male and 10 female rats received deionized water by gavage. All rats survived until the end of the study. The final mean body weights of 300 and 1,000 mg/kg males and of all dosed groups of females were significantly less than those of the vehicle controls. Erythrocyte microcytosis was demonstrated by decreased mean cell volumes in 300 mg/kg males and 1,000 mg/kg males and females. There was evidence of a thrombocytosis at all time points, demonstrated by increased platelet counts in the 100 mg/kg or greater groups. The serum activities of alanine aminotransferase and sorbitol dehydrogenase were increased in the 100 mg/kg or greater rats at various time points, suggesting hepatocellular injury. Additionally, bile acid concentrations were generally increased in the 300 and 1,000 mg/kg groups at all time points, consistent with cholestasis or altered hepatic function. A hypoproteinemia and hypoalbuminemia, evidenced by decreased total protein and albumin concentrations, occurred in rats in the 300 and 1,000 mg/kg groups at all time points.

Liver weights of 100, 300, and 1,000 mg/kg males and 300 and 1,000 mg/kg females and testis weights of 1,000 mg/kg males were significantly increased. Increased incidences of liver lesions occurred in 300 and 1,000 mg/kg males and females and hepatocellular adenoma occurred in one 1,000 mg/kg male. The incidences of atrophy and chronic inflammation of the mucosa of the glandular stomach were significantly increased in rats administered 300 or 1,000 mg/kg. Increased incidences of adrenal gland cortical hypertrophy and/or cytoplasmic alteration in the submandibular gland occurred in the 100 mg/kg or greater groups.

14-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice received methyleugenol in 0.5% methylcellulose by gavage at doses of 0, 10, 30, 100, 300, or 1,000 mg/kg, 5 days per week for 14 weeks. A water control group of 10 male and 10 female mice received deionized water by gavage. All but one male and all females receiving 1,000 mg/kg died before the end of the study. The mean body weight gains of mice in the 300 mg/kg groups were significantly less than those of the vehicle controls. The only clinical finding was toxicity manifested as generalized morbidity in mice administered 1,000 mg/kg. Liver weights of 30, 100, and 300 mg/kg males and of 300 mg/kg females were significantly increased. Male mice administered 10 or 30 mg/kg had significantly lower cauda epididymis, epididymis, and testis weights; males receiving 100 mg/kg had significantly lower spermatozoal concentrations. Increased incidences of liver lesions occurred in 1,000 mg/kg males and 300 and 1,000 mg/kg females. The incidences of lesions of the glandular stomach were increased in one or more groups administered 30 mg/kg or greater.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats received methyleugenol in 0.5% methylcellulose by gavage at doses of 37, 75, or 150 mg/kg, 5 days per week for 105 weeks; groups of 60 male and 60 female rats received the 0.5% methylcellulose vehicle only. Stop-exposure groups of 60 male and 60 female rats received 300 mg/kg in 0.5% methylcellulose by gavage for 52 weeks followed by just the 0.5% methylcellulose vehicle for the remaining 53 weeks of the study. Special study groups of 10 male and 10 female rats administered 36, 75, 150, or 300 mg/kg were designated for toxicokinetic studies.

Survival and Body Weights

All 150 and 300 mg/kg males died before the end of the study, and survival of 150 mg/kg females was slightly less than that of the vehicle controls. Mean body weights of all dosed groups of rats were less than those of the vehicle controls throughout most of the 2-year study.

Pathology Findings

Chemical-related liver neoplasms occurred in all dosed groups of rats and included hepatocellular adenoma, hepatocellular carcinoma, hepatocholangioma, and hepatocholangiocarcinoma; at 2 years, there were positive trends in the incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) in core study rats and in the numbers of rats with multiple liver neoplasms. Nonneoplastic lesions included eosinophilic and mixed cell foci, hepatocellular hypertrophy, oval cell hyperplasia, cystic degeneration, and bile duct hyperplasia (females); the incidences of these lesions in dosed groups of male and female rats were increased at 6 months, 12 months, and/or 2 years.

Chemical-related neoplasms and nonneoplastic lesions of the glandular stomach included benign and malignant neuroendocrine tumors in the 150 and 300 mg/kg groups and females in the 75 mg/kg group. In all dosed groups of rats at all time points, the incidences of mucosal atrophy were significantly greater than in the vehicle controls. Neuroendocrine cell hyperplasia was observed in females at 6 months and males and females at 12 months and at 2 years. In core study female rats, there was a positive trend in the incidences of squamous cell papilloma or carcinoma (combined) of the forestomach, and the incidence in the 150 mg/kg group exceeded the historical control range.

The incidences of renal tubule proliferative lesions in male rats were suggestive of a neoplastic effect in the kidney. Therefore, additional step sections of the kidneys of male rats were prepared. The incidences of renal tubule hyperplasia and adenoma in the extended evaluation and the combined incidences of standard and step sections in the 75, 150, and 300 mg/kg

groups were greater than those in the vehicle controls. The incidences of nephropathy were increased in all dosed groups of females, and the increase was significant in the 300 mg/kg group.

In dosed groups of male rats, there was a positive trend in the incidences of malignant mesothelioma, and the incidences were significantly greater in 150 and 300 mg/kg males than in the vehicle controls. The incidences of mammary gland fibroadenoma in 75 and 150 mg/kg males were significantly increased. The incidences of fibroma of the subcutaneous tissue in 37 and 75 mg/kg males and the combined incidences of fibroma or fibrosarcoma in 37, 75, and 150 mg/kg males were significantly increased.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice received methyleugenol in 0.5% methylcellulose by gavage at doses of 0, 37, 75, or 150 mg/kg for 105 weeks. Special study groups of 10 male and 10 female mice administered 37, 75, or 150 mg/kg were designated for toxicokinetic studies.

Survival and Body Weights

Survival of all dosed groups of male mice was similar to that of the vehicle controls. Survival of dosed groups of females was significantly less. Mean body weights of dosed mice were generally less than those of the vehicle controls throughout the studies.

Pathology Findings

Chemical-related increases in the incidences of liver neoplasms and nonneoplastic lesions in mice included hepatocellular adenoma and carcinoma, hepatoblastoma, hepatocholangiocarcinoma, eosinophilic foci, oval cell hyperplasia, bile duct hyperplasia, hemosiderin pigmentation, chronic active inflammation, and hematopoietic cell proliferation. In all dosed groups of males and females, the incidences of hepatocellular neoplasms and the multiplicity of neoplasms were generally greater than in the vehicle controls. The incidences of hepatoblastoma were significantly increased in all dosed groups of females and slightly increased in 150 mg/kg males. Hepatocholangiocarcinoma was observed in 150 mg/kg females. The incidences of eosinophilic foci, oval cell hyperplasia, portal hypertrophy, hepatocyte necrosis, hematopoietic cell proliferation, bile duct hyperplasia, and hemosiderin pigmentation were significantly increased in two or more dosed groups of male and/or female mice.

The incidences of glandular ectasia, mucosal atrophy, chronic active inflammation, epithelial hyperplasia, and neuroendocrine cell hyperplasia of the glandular stomach were increased in one or more dosed groups of male and female mice. In addition, malignant neuroendocrine tumors were observed in the glandular stomach of two 150 mg/kg male mice; one male in this group had a carcinoma.

TOXICOKINETIC STUDIES

Methyleugenol is rapidly absorbed following oral administration to rats and mice. The kinetic data are consistent with rapid clearance from the blood, metabolism in the liver, and excretion of the parent and various metabolites in the urine.

GENETIC TOXICOLOGY

Methyleugenol was not mutagenic in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation (S9). In cytogenetic tests with cultured Chinese hamster ovary cells, methyleugenol induced sister chromatid exchanges in the presence of S9, but no induction of chromosomal aberrations was noted in cultured Chinese hamster ovary cells following exposure to methyleugenol, with or without S9. *In vivo*, no increase in the frequency of micronucleated normochromatic erythrocytes was seen in male or female mice administered methyleugenol by gavage for 14 weeks.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

A physiologically based pharmacokinetic (PBPK) model resulting from intravenous and oral exposure was created to characterize tissue concentrations of methyleugenol in rats and mice. Data used to create the model were obtained from the literature or from current studies. The primary conclusions that can be reached from the PBPK model are: 1) absorption of oral doses of methyleugenol in rats and mice is rapid and complete, 2) distribution of methyleugenol to

tissues is not hampered by capillary permeability, and 3) metabolism of methyleugenol is saturable and must have some extrahepatic component in the mouse. Model-based plasma methyleugenol concentrations were not found to be good dosimeters for evaluating neoplasm dose-response data.

CONCLUSIONS

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** of methyleugenol in male and female F344/N rats based on the increased incidences of liver neoplasms and neuroendocrine tumors of the glandular stomach in male and female rats and the increased incidences of kidney neoplasms, malignant mesothelioma, mammary gland fibroadenoma, and subcutaneous fibroma and fibroma or fibrosarcoma (combined) in male rats. A marginal increase in the incidence of squamous cell neoplasms of the forestomach may have been related to methyleugenol administration in female rats. There was *clear evidence of carcinogenic activity* of methyleugenol in male and female $B6C3F_1$ mice based on the increased incidences of liver neoplasms. Neuroendocrine tumors of the glandular stomach in male mice were also considered related to methyleugenol administration.

In male and female rats and mice, methyleugenol administration caused significant increases in the incidences of nonneoplastic lesions of the liver and glandular stomach.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 16.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Methyleugenol

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses in methylcellulose by gavage	0, 37, 75, or 150 mg/kg or 300 mg/kg (stop-exposure)	0, 37, 75, or 150 mg/kg or 300 mg/kg (stop-exposure)	0, 37, 75, or 150 mg/kg	0, 37, 75, or 150 mg/kg
Body weights	Dosed groups less than the vehicle control group	Dosed groups less than the vehicle control group	Dosed groups less than the vehicle control group	Dosed groups less than the vehicle control group
Survival rates	20/50, 16/50, 15/50, 0/50, 0/50	22/50, 25/50, 22/50, 11/50, 16/50	38/49, 36/50, 37/50, 35/50	31/50, 18/50, 18/50, 2/50
Nonneoplastic effects	Liver: eosinophilic foci (11/50, 28/50, 43/50, 47/50, 39/50); mixed cell foci (1/50, 7/50, 14/50, 8/50, 2/50); hepatocyte hypertrophy (0/50, 13/50, 25/50, 30/50, 26/50); oval cell hyperplasia (14/50, 17/50, 24/50, 34/50, 27/50); cystic degeneration (4/50, 2/50, 25/50, 38/50, 41/50) Glandular stomach: neuroendocrine cell hyperplasia (0/50, 0/50, 1/50, 8/50, 8/50); atrophy (0/50, 14/50, 32/50, 37/50, 29/50)	Liver: eosinophilic foci (10/50, 20/50, 27/49, 31/49, 37/50); mixed cell foci (6/50, 4/50, 19/49, 9/49, 7/50); hepatocyte hypertrophy (1/50, 13/50, 16/49, 26/49, 31/50); oval cell hyperplasia (1/50, 15/50, 19/49, 35/49, 34/50); bile duct hyperplasia (11/50, 11/50, 17/49, 22/49, 30/50); cystic degeneration (0/50, 0/50, 1/49, 4/49, 29/50) Glandular stomach: neuroendocrine cell hyperplasia (0/50, 5/50, 11/50, 9/50, 3/50); atrophy (3/50, 41/50, 45/50, 39/50, 33/50)	Liver: eosinophilic foci (10/49, 20/50, 25/50, 19/50); oval cell hyperplasia (0/49, 8/50, 27/50, 46/50); hepatocyte hypertrophy (0/49, 1/50, 7/50, 46/50) Glandular stomach: atrophy (0/49, 3/48, 35/49, 45/50); hyperplasia (0/49, 1/48, 15/49, 20/50); ectasia (13/49, 25/48, 40/49, 49/50)	Liver: oval cell hyperplasia (0/50, 46/50, 36/49, 38/50); hepatocyte hypertrophy (0/50, 10/50, 7/49, 23/50); hepatocyte necrosis (5/50, 9/50, 16/49, 17/50); hematopoietic cell proliferation (4/50, 14/50, 23/49, 24/50); bile duct hyperplasia (1/50, 1/50, 11/49, 9/50); hemosiderin pigmentation (0/50, 11/50, 24/49, 19/50) Glandular stomach: atrophy (0/45, 0/49, 10/46, 10/45); ectasia (14/45, 33/49, 31/46, 38/45)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Methyleugenol

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Liver: hepatocellular adenoma (5/50, 12/50, 23/50, 38/50, 32/50); hepatocellular carcinoma (2/50, 3/50, 14/50, 25/50, 36/50); hepatocellular adenoma or carcinoma (7/50, 14/50, 28/50, 43/50, 45/50); hepatocholangioma (0/50, 0/50, 0/50, 1/50, 6/50); hepatocholangiocarcinoma (0/50, 0/50, 1/50, 1/50, 7/50); hepatocholangioma or hepatocholangiocarcinoma (0/50, 0/50, 1/50, 2/50, 13/50) Glandular stomach: benign neuroendocrine tumor (0/50, 0/50, 0/50, 3/50, 2/50); malignant neuroendocrine tumor (0/50, 0/50, 0/50, 4/50, 2/50); benign or malignant neuroendocrine tumor (0/50, 0/50, 0/50, 7/50, 4/50) Kidney: renal tubule adenoma (standard and extended evaluations combined - 4/50, 6/50, 17/50, 13/50, 20/50) Malignant mesothelioma: (1/50, 3/50, 5/50, 12/50, 5/50) Mammary gland: fibroadenoma (5/50, 5/50, 15/50, 13/50, 6/50) Skin (subcutaneous): fibroma (1/50, 9/50, 8/50, 5/50, 4/50); fibroma or fibrosarcoma (1/50, 12/50, 8/50, 8/50, 4/50)	Liver: hepatocellular adenoma (1/50, 8/50, 11/49, 33/49, 43/50); hepatocellular carcinoma (0/50, 0/50, 4/49, 8/49, 22/50); hepatocellular adenoma or carcinoma (1/50, 8/50, 14/49, 34/49, 43/50); hepatocholangioma (0/50, 0/50, 0/49, 0/49, 8/50); hepatocholangiocarcinoma (0/50, 0/50, 0/49, 3/49, 9/50); hepatocholangioma or hepatocholangiocarcinoma (0/50, 0/50, 0/49, 3/49, 17/50) Glandular stomach: benign neuroendocrine tumor (0/50, 0/50, 13/50, 9/50, 5/50); malignant neuroendocrine tumor (0/50, 1/50, 12/50, 26/50, 36/50); benign or malignant neuroendocrine tumor (0/50, 1/50, 25/50, 34/50, 41/50)	Liver: hepatocellular adenoma (26/49, 43/50, 38/50, 39/50); hepatocellular carcinoma (10/49, 20/50, 19/50, 9/50); hepatocellular adenoma or carcinoma (31/49, 47/50, 46/50, 40/50); hepatoblastoma (0/49, 0/50, 1/50, 3/50) Glandular stomach: malignant neuroendocrine tumor (0/49, 0/48, 0/49, 2/50)	Liver: hepatocellular adenoma (20/50, 48/50, 46/49, 41/50); hepatocellular carcinoma (7/50, 37/50, 47/49, 47/50); hepatocellular adenoma or carcinoma (25/50, 50/50, 49/49, 49/50); hepatoblastoma (0/50, 6/50, 11/49, 15/50); hepatocholangiocarcinoma (0/50, 0/50, 0/49, 2/50)
Uncertain findings		Forestomach: squamous cell papilloma or carcinoma (0/50, 0/50, 1/50, 3/50, 1/50)		
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Methyleugenol

Genetic toxicology	
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537, with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Positive with S9, negative without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on methyleugenol on 30 October 1998 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Gary P. Carlson, Ph.D., Chairperson
School of Health Sciences
Purdue University
West Lafayette, IN

A. John Bailer, Ph.D.
Department of Mathematics and Statistics
Miami University
Oxford, OH

Steven A. Belinsky, Ph.D.
Inhalation Toxicology Research Institute
Kirkland Air Force Base
Albuquerque, NM

James S. Bus, Ph.D.
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Linda A. Chatman, D.V.M.*
Pfizer, Inc.
Groton, CT

John M. Cullen, V.M.D., Ph.D.
Department of Microbiology, Parasitology, and Pathology
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Susan M. Fischer, Ph.D.*
M.D. Anderson Cancer Center
The University of Texas
Smithville, TX

Thomas L. Goldsworthy, Ph.D.*
Integrated Laboratory Systems, Inc.
Research Triangle Park, NC

Stephen S. Hecht, Ph.D.
University of Minnesota Cancer Centers
Minneapolis, MN

Michele Medinsky, Ph.D.
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Jose Russo, M.D.*
Fox Chase Cancer Center
Philadelphia, PA

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 30 October 1998, the draft Technical Report on the toxicology and carcinogenesis studies of methyleugenol received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of methyleugenol by discussing the uses of the chemical, describing the rationale for the study and the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year gavage studies of methyleugenol were *clear evidence of carcinogenic activity* in male and female F344/N rats and $B6C3F_1$ mice.

Dr. R.A. Herbert, NIEHS, characterized the lesions in the fundic region of the glandular stomach associated with methyleugenol administration in male and female rats and mice. These lesions included atrophy and neuroendocrine cell hyperplasia, as well as benign and malignant neuroendocrine tumors, which are rare in rats and mice as either spontaneous or chemically induced lesions. Dr. Herbert described a series of short-term (14-, 30-, and 90-day) studies providing data that supported the hypothesis that parietal cell cytotoxicity with subsequent mucosal atrophy, increased intragastric pH, and increased circulating gastrin (hypergastrinemia) is probably how methyleugenol produces neuroendocrine tumors in the glandular stomach.

Dr. M.L. Cunningham, NIEHS, presented data from work in progress that described *in vivo* and *in vitro* studies of methyleugenol metabolism in rodents and some recent results from human model systems. He began by describing the more widely studied metabolism of the close structural analogue and hepatocarcinogen, safrole, and contrasted the results with those obtained for methyleugenol. The findings to date indicate that methyleugenol can undergo a variety of Phase 1 oxidation reactions, that metabolites can be further metabolized through Phase 2 conjugations to yield reactive sulfonoxy metabolites, and that human tissue preparations are capable of metabolizing and bioactivating the chemical. The genetic toxicity of methyleugenol is similar to safrole and for both compounds appears to be dependent on both Phase 1 and Phase 2 metabolic activation.

Dr. T.R. Devereux, NIEHS, provided information on molecular alterations in neoplasms from the NTP study, concentrating on the mouse liver and lung neoplasms for which there is a large database of genetic information. She focused on the APC/β-catenin-Wnt signaling pathways that have been implicated in various human and rodent cancers. In neoplasm cells, either a mutation in the APC gene or in β-catenin can upregulate β-catenin and the Wnt signaling pathway, leading eventually to cell proliferation. β-Catenin mutations were found in about half of the methyleugenol mouse liver neoplasms compared with mutations in only 5% of spontaneous neoplasms. Mutations were found at the same sites as those in human hepatocellular carcinomas, suggesting similar carcinogenic pathways. Genetic alterations were not found in H-*ras* or p53, suggesting that these genes are not involved in methyleugenol-induced mouse liver carcinogenesis.

Dr. G.M. Blumenthal, NIEHS, discussed the development of physiologically based pharmacokinetic models to describe and simulate the toxicokinetics of methyleugenol in rats and humans. Animal data were obtained from single-dose administration to rats at 37 mg/kg by intravenous injection and by gavage at 37, 75, and 150 mg/kg. Human data were obtained from an in-house study in which volunteers ate 12 gingersnaps; blood samples were collected prior to exposure and 15, 30, 60, and 120 minutes afterward. Data was also obtained from the NHANES database collected by the Centers for Disease Control and Prevention. The studies to date show that absorption of methyleugenol was rapid in rats and humans with a large first pass effect in rats that was also assumed in humans. Metabolism was saturated at all doses in rats, while a slower metabolism was predicted in humans. Over 90% of the doses were metabolized within 24 hours in rats, and this value was assumed for the human model. More studies are in process and should lead to an entire dose response characterization.

Dr. Hecht, a principal reviewer, agreed with the proposed conclusions. He wondered why, considering the structural similarity to safrole and human exposure to methyleugenol, the NTP had not studied this chemical earlier.

Dr. Cullen, the second principal reviewer, agreed with the proposed conclusions. He thought the study was remarkable because of the presence of two unusual neoplasms. In the liver, unusual mixed neoplasms composed of cholangiocellular and hepatocellular elements suggest a potent carcinogenic effect that affects both biliary and hepatic cell lineage or, possibly, a stem-cell population. He said that gastric neuroendocrine tumors are also rare and thought it prudent that immunohistochemical and histochemical stains were done to establish the cell type. Dr. Herbert noted that some of the liver neoplasms appeared to have a hepatocellular component and a biliary cell component; therefore, the diagnoses of hepatocholangiocellular neoplasms were most descriptive. Dr. Cullen said the dose-related increases in the incidences of oval cell hyperplasia in mice suggested further discussion of the possibility of a synergistic effect with the presence of *Helicobacter* and this lesion.

Dr. Bus, the third principal reviewer, agreed with the proposed conclusions. He complimented the NTP on the extensive toxicokinetic and disposition studies including information on how disposition may change with time and with the age of the animals. Noting that the low dose in the rodent studies, 37 mg/kg, was metabolically saturating and likely not a no-observed-effect level, Dr. Bus suggested there were lessons here for future protocol designs to provide data more valuable for future risk assessment purposes. Dr. J.R. Bucher, NIEHS, commented that methyleugenol is listed as a "generally recognized as safe" substance in the United States, and although there is a 5 mg/kg limit in Europe, there are not large differences between concentrations permitted in foods and the 37 mg/kg dose used in rats and mice. Palatability may be the limiting factor.

Dr. Medinsky cautioned that bioavailability of a compound is less relevant when a metabolite is the active/toxic form. Dr. Bailer commented that he was a bit uncomfortable with the possible utility of data for risk assessment purposes when the lowest animal dose is approximately 37,000 times the human dose (the gingersnap study). Dr. G.W. Lucier, NIEHS, pointed out that the blood levels from the NHANES study were only about 1,000-fold greater than the rat blood levels.

Dr. Tim Adams, Flavor and Extract Manufacturers Association (FEMA), stated that actual exposure to methyleugenol has substantially decreased over the last 30 years, with most coming from fruits and spices. Further, FEMA estimates that exposure in the diet exceeds intentional addition by a factor of at least 100. With regard to the neuroendocrine lesions in the stomach, he noted that the agent was given by gavage in a microencapsulated form, perhaps allowing for prolonged stomach exposure. Dr. Bucher said the methyleugenol was in methylcellulose, the gavage vehicle, and not microencapsulated.

Dr. Hecht moved that the Technical Report on methyleugenol be accepted with revisions discussed and the conclusions as written for male and female rats and mice, *clear evidence of carcinogenic activity*. Dr. Cullen seconded the motion, which was accepted unanimously with six votes.